DOCKET NO.: WARF-0135 (P00154US) Application No.: 09/651,290 Office Action Dated: July 12, 2002

REPLY FILED UNDER EXPEDITED PROCEDURE PURSUANT TO 37 CFR § 1.116

PATENT

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. (Amended) A recombinant bacterium, which is An antibacterial agent, which eemprises a non-pathogenic conjugative donor bacterium bacterial cell harboring at least one transmissible plasmid comprising:

a) an origin of replication for synthesizing the plasmid in a bacterial cell, wherein initiation of replication at the origin is negatively controlled by a plasmid replication repressor, wherein in the absence of the plasmid replication repressor the transmissible plasmid undergoes runaway replication;

b) an origin of transfer from which conjugative transfer of the transmissible plasmid initiates from the donor cell to at least one recipient cell; and, optionally,

c) at least one screenable marker gene;

wherein the donor cell further comprises one or more transfer genes conferring upon the donor cell the ability to conjugatively transfer the transmissible plasmid to the recipient cell, and wherein the donor cell produces the plasmid replication repressor, and further wherein the at least one recipient cell is a pathogenic bacterium that does not produce the plasmid replication repressor, thereby enabling the transmissible plasmid to undergo runaway replication in the recipient cell.

2. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 1, wherein the transfer genes are contained on a helper plasmid within the donor cell, such that the transmissible plasmid is transmissible from the donor cell to a recipient cell, but is not further self-transmissible from the recipient cell to another recipient cell.

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3. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 1, wherein the transfer genes are contained on the transmissible plasmid, such that the transmissible plasmid is self-transmissible from the donor cell to a recipient cell, and further from the recipient cell to another recipient cell.

4. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 1, wherein the transmissible plasmid comprises a derivative of a naturally-occurring transmissible plasmid containing a gene encoding the replication repressor that has been mutated to produce a non-functional replication repressor.

- 5. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 4, wherein the naturally-occurring transmissible plasmid is selected from the group consisting of RK2, R6K, pCU1, p15A, pIP501, pAMβ1 and pCRG1600.
- 6. (Amended) The recombinant bacterium antibacterial agent of claim 5, wherein the naturally-occurring plasmid is R6K and the mutation comprises a mutation in the R6K pir gene such that its encoded π protein comprises an at least one amino acid deletion or substitution at amino acids 105, 106 or 107.
- 7. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 1, wherein the donor cell is a non-pathogenic strain of bacteria selected from the group consisting of

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Escherichia coli, Lactobacillus spp., Lactococcus, Bifidobacteria, Eubacteria, and bacterial minicells.

- 8. (Amended) The <u>recombinant bacterium antibacterial agent</u> of claim 1, wherein the recipient cell is a pathogenic strain of bacterium selected from the group consisting of Campylobacter spp., Enterobacter spp., Enterococcus spp., Escherichia coli, Gardnerella vaginalis, Haemophilus spp., Helicobacter pylorii, Mycobacterium tuberculosis, Propionobacter acnes, Pseudomonas aeruginosa and other Pseudomonas spp., Salmonella typhimurium, Shigella spp. and Staphylococcus spp.
- 9. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 1, wherein the origin of replication is derived from a plasmid selected from the group consisting of R6K, RK2, rts1, p15A and RSF1010.
- 10. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 1, wherein the origin of replication is selected from the group consisting of F and P1.
- 11. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 1, wherein the screenable marker gene confers a nutritional selection advantage on cells containing the transmissible plasmid.

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12. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 1, wherein the transfer genes are derived from a plasmid selected from the group consisting of F, R6K and Ti.

- 13. Canceled
- 14. Canceled
- 15. Canceled
- 16. (Amended) An <u>recombinant bacterium</u> antibacterial agent, which comprises a non-pathogenic donor bacterial cell harboring at least one transmissible plasmid comprising:
 - a) an origin of replication for synthesizing the plasmid in a bacterial cell;
- b) an origin of transfer from which conjugative transfer of the transmissible plasmid initiates from the donor cell to at least one recipient cell;
- c) at least one killer gene that, upon expression in a bacterial cell, produces a product that kills the cell; and, optionally,
 - d) at least one screenable marker gene;

wherein the donor cell further comprises one or more transfer genes conferring upon the donor cell the ability to conjugatively transfer the transmissible plasmid to the recipient cell, and wherein the donor cell is modified so as to be unaffected by the product of the killer gene, and further wherein the at least one recipient cell is a pathogenic bacterium that has not been modified so as to be unaffected by the product of the killer gene.

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17. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 16, wherein the transfer genes are contained on a helper plasmid within the donor cell, such that the transmissible plasmid is transmissible from the donor cell to a recipient cell, but is not further self-transmissible from the recipient cell to another recipient cell.

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18. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 16, wherein the transfer genes are contained on the transmissible plasmid, such that the transmissible plasmid is self-transmissible from the donor cell to a recipient cell, and further from the recipient cell to another recipient cell.

- 19. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 16, wherein the killer gene kills the cells by being expressed and thereby producing a gene product that is detrimental or lethal to bacterial cells, and the donor cells have been modified so as to repress the expression of the killer gene, thereby avoiding production of the detrimental or lethal gene product.
- 20. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 16, wherein the killer gene is obtained from a bacteriophage.
- 21. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 20, wherein the bacteriophage is selected from the group consisting of T-series phages, P1, p22 and λ .

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22. (Amended) The <u>recombinant bacterium</u> antibacterial agent] of claim 16, wherein the donor cell is a non-pathogenic strain of bacteria selected from the group consisting of *Escherichia coli*, *Lactobacillus spp.*, *Lactococcus*, *Bifidobacteria*, *Eubacteria*, and bacterial minicells.

- 23. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 16, wherein the recipient cell is a pathogenic strain of bacterium selected from the group *Campylobacter spp.*, Enterobacter spp., Enterococcus spp., Escherichia coli, Gardnerella vaginalis, Haemophilus spp., Helicobacter pylorii, Mycobacterium tuberculosis, Propionobacter acnes, Pseudomonas aeruginosa and other Pseudomonas spp., Salmonella typhimurium, Shigella spp. and Staphylococcus spp.
- 24. (Amended) The recombinant <u>recombinant bacterium</u> antibacterial agent of claim 16, wherein the origin of replication is derived from a plasmid selected from the group consisting of R6K, RK2, rts1, p15A and RSF1010.
- 25. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 16, wherein the origin of replication is selected from the group consisting of F and P1.
- 26. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 16, wherein the screenable marker gene confers a nutritional selection advantage on cells containing the transmissible plasmid.

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27. (Amended) The <u>recombinant bacterium</u> antibacterial agent of claim 16, wherein the transfer genes are derived from a plasmid selected from the group consisting of F, R6K and Ti.

- 28. Canceled
- 29. Canceled
- 30. Canceled